REMARKS

Claim Amendments

New claim 79 is provided herewith, and claims 44, 60, 77, and 78 are amended herewith, without disclaimer of subject matter and solely to advance prosecution. These amendments present rejected claims in better form for allowance or consideration on appeal and accordingly Applicants respectfully request their entry. As was discussed in a telephonic interview, these amendments obviate several of the rejections. Specifically, the amendment of claims 44, 60, and 78 to recite "chymosin from a bovine ~~or *Camelidae*~~ species <u>or *Camelus dromedarius*</u>" conforms to the scope indicated to have adequate written description support in the Office Action. Support for this amendment is found in at least original claim 29. Similarly, the amendment to claim 77 to recite "wherein the glucoamylase is derived from <u>culture of</u> an *Aspergillus* species" eliminates susceptibility to the interpretation that the Examiner adopted in making the rejections. Support for this amendment is found in at least original claim 26.

Additionally, claim 44 is amended to remove the recitation "lactic acid" from the Markush group and instead present it in a separate independent claim (which is new claim 79 in this paper). As discussed in the telephonic interview, the presentation of lactic acid in a separate independent claim is intended to place the application in better condition for allowance or appeal because lactic acid raises different issues owing to its lower pK_a than acetic acid. Support for this claim is found as stated previously with claim 44 (see Applicant's remarks dated July 20, 2010, pg. 6). Applicants further note that new claim 79 does not raise any additional issues that would preclude its entry because lactic acid was previously recited in claim 44 and has been particularly addressed in the prior office action.

Since these amendments place the claims in better condition for appeal and do not raise new issues, their entry after a Final Rejection is permissible. *See* MPEP 714.12 and 37 C.F.R. § 1.116(b)(2). Accordingly Applicants respectfully request entry of these amendment.

No new matter has been added by these amendments.

Interview Summary

Applicants are grateful for the telephonic interview conducted on October 14, 2010. Present on the call were Examiner Steadman and Applicant's representatives Robin Teskin and

Ken Kalafus. During the interview, Applicants described proposed claim amendments intended to obviate the majority of the issues in the application. In claims 44, 60, and 78, Applicants proposed amending the claims to recite "a gene encoding chymosin from a bovine ~~or *Camelidae*~~ species or *Camelus dromedarius*" which had been indicated in the Office Action to have sufficient written description support. Additionally, Applicants proposed to amend claim 77 to recite "wherein the glucoamylase is derived from culture of an *Aspergillus* species," which is not susceptible to the interpretation that the Examiner adopted in making the written description, enablement, and new matter rejections. These amendments are reflected in the listing of the claims provided herewith.

The remaining issue, obviousness, was also discussed. Central to each rejection is Lawlis reference (U.S. Patent No. 5,801,034). Lawlis discloses a method of killing cells by adding an organic acid and lowering the pH of the medium. The pH used in the Lawlis method differs depending on the particular organic acid chosen, and the rejections have been differently stated depending on the acid involved (acetic acid, lactic acid, and formic acid). Because these three acids raise different issues, Applicants proposed to remove the recitation "lactic acid" from the Markush group in claim 44 and instead present it in a separate independent claim (which is new claim 79 in this paper). Applicants explained that these amendments amendment would place the application in better condition for allowance or appeal because the obviousness rejection of each independent claim would only relate to a single organic acid: acetic acid (claims 44 and 78), formic acid (claim 60), or lactic acid (new claim 79). Applicants then described their arguments traversing the obviousness rejections, which are presented below.

Response to rejections concerning Acetic Acid

Claims 44-46, 50-55, 58-59 and 78 have been rejected as allegedly obvious over Lawlis in view of Ward (Office Action, page 26), and claim 57 has been rejected over these references and in further view of EMBL AJ131677 (Office Action, page 30). Lawlis teaches a method of killing cells without lysis. The Lawlis method simplifies recovery and purification of secreted enzymes because the intact cells are easier to remove from the media than the cytoplasmic contents that would be released from lysed cells. It is undisputed that Lawlis only teaches a method of killing cells, and does not provide any guidance for selection of pH values that would achieve the beneficial result recited in the present claims, namely inactivating unwanted

glucoamylase activity while maintaining chymosin activity. Notwithstanding these differences, the Examiner has alleged that it would have been obvious to use the method of Lawlis, with acetic acid, and within the range of pH values recited in the present claims, to kill the cells taught by Ward. Applicants respectfully traverse and request reconsideration and withdrawal of the rejection for the reasons stated in their previous response and for the further reasons described below.

Though Lawlis does claim an open-ended range of pH values, the reference does not justify lowering the pH to between 1.8 and 1.0 as recited in claims 44 and 78 (or any of the sub-ranges that are recited in the dependent claims). Rather, Lawlis demonstrates complete cell killing with acetic acid at pH higher than the values recited in the claims. Specifically, in examples 1 and 2, no viable cells remained after treatment with acetic acid at pH 2.0. Because Lawlis only teaches using acidic treatment to kill cells and this goal is completely achieved with acetic acid at pH 2.0, Lawlis does not justify lowering the pH to between 1.8 and 1.0 as recited in claims 44 and 78.

The mechanism of cell killing also does not provide any justification for lowering the pH to within the claimed range. Rather, the mechanism of action indicates that further lowering of pH would be inconsequential. That mechanism of action is as follows:

> By reducing the pH of the mixture or media to a value equal to or less than two pH units below the pK_a of the organic acid to be used, the acid is 99% protonated or uncharged and becomes "invisible" to the cell as an acid. The cell may then take up or import the neutral acid compound in the usual manner as a nutrient. . . . which kills the cell.

Lawlis, col. 4, lines 13-21. Based on this mechanism of action the Examiner has alleged that Lawlis teaches that pH is a result-effective variable for achieving cell kill because the percentage of active form could be slightly increased with lowered pH (Office Action, page 27) and has concluded that one of ordinary skill in the art would have been motivated to lower the pH farther than 2 units below the pK_a to achieve a more complete cell kill. *However, Lawlis teaches that organic acid concentration, rather than pH, is the critical result-effective variable* once the pH has been lowered to pK_a - 2. This is because once the pH is lowered to pK_a - 2, the organic acid is already 99% in the active form. Even the most extreme lowering of pH (*e.g.*, adding an excess

volume of fuming hydrochloric acid) could only increase the active acid concentration by about 1%. In contrast the active acid concentration can readily be doubled, tripled, or even further increased (relative to the concentrations in the working examples) by simply increasing the organic acid concentration, because the active acid concentration increases proportionately to the total organic acid concentration. Thus, once the pK_a - 2 threshold has been reached, the organic acid concentration is the critical result-effective variable. Accordingly, contrary to the alleged basis of rejection, one would not be motivated to increase cell killing by lowering the pH below pK_a - 2 because this would be ineffective; rather, if greater cell killing were desired then the organic acid concentration would be increased without altering pH.

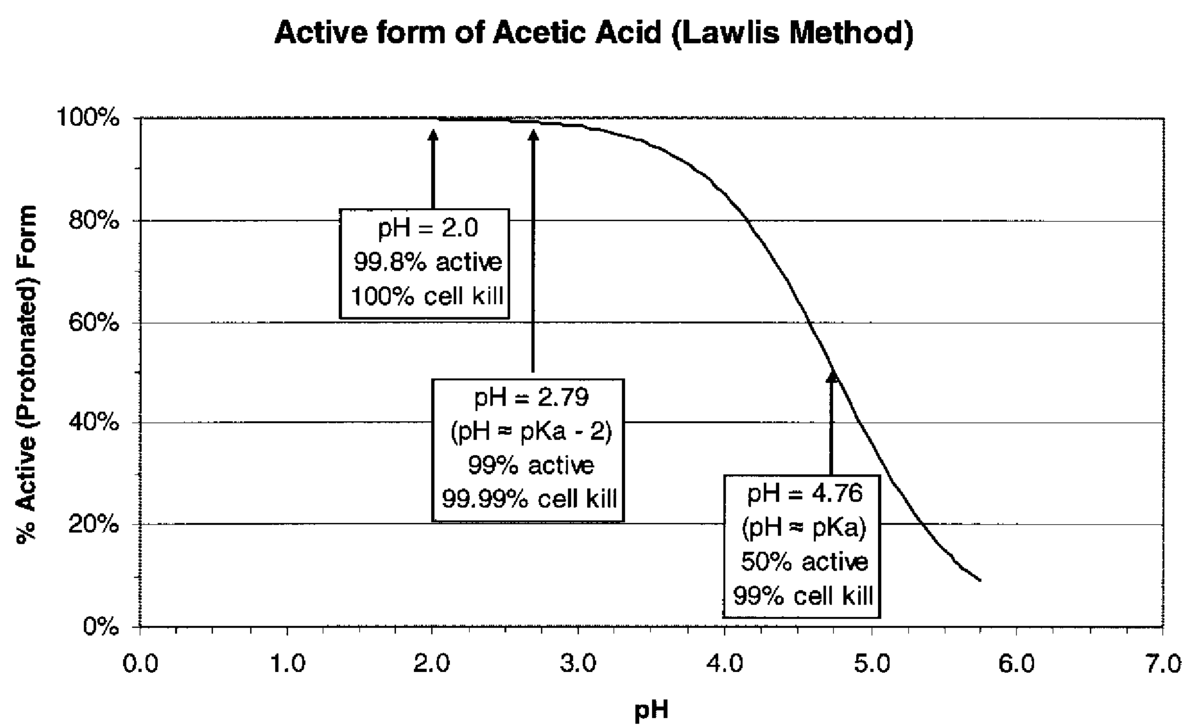

Figure 1. Percent active (uncharged) form of acetic acid as a function of pH. Percentage active form was computed using the Henderson–Hasselbalch equation, $pH = pK_a + \log([A^-]/[HA])$. Cell kill percentages shown in the callouts are from Examples 1, 2, and 3 in Lawlis (percentages were computed by comparing the cell counts of the treated samples to the control samples, using the dilution having the highest number of counted cells for each sample).

The insignificance of lowering the pH below pK_a - 2 is illustrated in Figure 1 above. As pH is lowered to 2.75 (two units below pK_a), the percentage of active form rapidly increases to

99%. Below this point, the curve flattens and the percentage of active form asymptotically approaches 100%. Though there may be some minor increase in the amount of acetic acid in the active form, Lawlis teaches that 99% active form is highly effective at cell killing and that further lowering of pH would have insignificant impact. For example, at pH 2.79 (*i.e.*, approximately equal to pK_a - 2), 99% of the acetic acid is in the active form, and 99.99% of the cells were killed in four hours (Example 3, Sample # 6). Though a longer overnight treatment at pH 2.0 (99.8% of acetic acid in active form) achieved 100% cell kill (examples 1 and 2), Lawlis does not suggest any preference for the pH 2.0 treatment, but apparently treats the additional 0.01% cell kill as either mathematically insignificant or attributable to the greater treatment duration (overnight instead of 4 hours). Thus, Lawlis does not express any preference for a pH lower than pK_a - 2, and both the empirical cell killing results and the mechanism of action do not suggest any benefit to lowering the pH below pK_a - 2.

The express teachings of Lawlis also do not support the Examiner's contention that it would have been obvious to lower the pH below pK_a - 2 to achieve greater cell killing. For example, Lawlis nowhere states that there would be any benefit to lowering pH below pK_a - 2. Rather, Lawlis only teaches that greater cell killing can be achieved with greater organic acid concentration. *See* Lawlis, col. 3, lines 38-45 (describing exemplary acetic acid concentrations sufficient to achieve cell killing); col. 3, lines 62-64 ("After the pH is adjusted to the proper level, the organic acid is added in an amount sufficient to effect the desired cell kill.") Moreover, increasing the organic acid concentration does not affect the pH in the Lawlis method; rather, the target pH is achieved by decreasing the amount of inorganic acid to offset any increase in organic acid concentration, or alternatively Lawlis teaches adding an organic acid salt to achieve the same effect as an organic acid without altering pH. *See* col. 3, line 65 to col. 4, line 9, col. 4, lines 28-38, and Example 3.

The Examiner has further alleged that one of ordinary skill in the art would have found it obvious to use a pH value of 1.75 because this pH value is mentioned in Lawlis and would recognize that this pH value is below pK_a - 2. Office Action, page 27. However, this pH value is only recited for use with formic acid, whose pK_a is 3.75.

> the pH of the culture or fermentation mixture is first adjusted using a mineral acid to a pH approximately equal to or less than about 2 pH units

> below the pK_a of the organic acid selected for use for the cell kill. For example, if formic acid (pK_a =3.75) is to be used to accomplish the cell kill, the pH of the mixture will be adjusted with a mineral acid to about 1.75 or less

Lawlis, col. 3, lines 56-60 (emphasis added). This is the only mention of pH 1.75 (or any pH value below 2.0) in the entire reference, and Lawlis explicitly states that this pH value is only chosen for formic acid because it is 2 pH units below the pK_a of that particular acid. Thus, contrary to the alleged basis of rejection, Lawlis does not teach use of pH 1.75 for any organic acid other than formic acid, but rather teaches that the pH value is to be selected based on the pK_a value of the organic acid. Formic acid has a different pK_a than acetic acid, and therefore the pH taught for formic acid is immaterial. Rather, since the pK_a of acetic acid is 4.75, the pH value taught for that acid is 2.75 (pK_a - 2).

Thus, where acetic acid is used (pK_a 4.75), Lawlis does not provide any justification for lowering the pH to any value lower than 2.75 (i.e., pK_a - 2). If greater cell killing is desired, Lawlis teaches increasing the acid concentration, but nowhere suggests any benefit to lowering the pH any lower than 2.75 when using acetic acid. The mechanism of cell killing also does not provide any justification for further lowering of the pH, since the critical result-effective variable is organic acid concentration once the pH has been lowered to pK_a - 2, and further lowering of pH is inconsequential. The working examples also illustrate complete cell kill at pH values higher than between 1.8 and 1.0 as recited in the claims; since the purpose of the Lawlis method (killing cells) is completely achieved at these pH values, there is no rationale for lowering the pH any further. Finally, the secondary references fail to remedy these deficiencies of Lawlis. Therefore, there is no justification for lowering the pH to any value between 1.8 and 1.0 when using acetic acid with the Lawlis method, and accordingly claims 44-46, 50-55, 57-59 and 78 are not obvious.

The pK_a of lactic acid is 3.86

The prior Office Action included an obviousness rejection based on the use of lactic acid with the Lawlis method. As discussed above, Lawlis teaches selecting the treatment pH based on the pK_a of the organic acid, thus the pK_a of lactic acid is of fundamental importance to the rejection. This pK_a value has been disputed during prosecution. Applicants have previously

provided an authoritative source (the 2006 Merck Index) showing the pK_a value of lactic acid to be 3.86. The Examiner had identified one patent (Van Ooijen, U.S. Patent No. 5,371,287) and now has identified one non-patent literature publication (Griffin *et al.*, Substrate-dependent proton load and recovery of stunned hearts during pyruvate dehydrogenase stimulation. Am J Physiol Heart Circ Physiol. 2000 Jul;279(1):H361-7) which state that the pK_a of lactic acid is 3.08. In weighing which reference teachings to adopt, the Examiner gave three reasons to reject the teachings of the 2006 Merck Index. First, the Examiner stated that the 2006 Merck Index indicated pK rather than pK_a values and did not indicate whether these were equivalent. Office Action, page 24. To address this concern, Applicants present Exhibit A which provides pages of the CRC Handbook of Chemistry and Physics (2003-2004 edition) which shows the pK_a of lactic acid is 3.86. Because this pK_a value is exactly the same as the "pK" value for DL-lactic acid in the 2006 Merck Index, it is apparent that these terms refer to the same constant.

Second, the Examiner has stated that because it was published after the present application's filing date, the 2006 Merck Index was "not relevant to establishing the known pK_a of lactic acid *at the time of invention*." Office Action, page 23 (emphasis in original). However, Applicants respectfully submit that the methods of measuring pK_a are firmly established in the art, such that it would be inconceivable for the accepted pK_a value to change so dramatically in just a few years, and thus a pK_a value reported in 2006 is representative of the contemporaneous understanding in the art on the date that the present application was filed. Nonetheless, to advance prosecution, Applicants present herewith Exhibit B which provides pages of the 1989 Merck Index providing the pK_a for DL-lactic acid is 3.86: the reference shows the equilibrium constant, K is 1.38×10^{-4} and states that $pK = 1 / \log_{10} K$ (*see* definitions of K and pK at pgs. xvi and xvii), which is 3.86. As noted above, the pK value for lactic acid furnished in the Merck Index is the same as the pK_a value reported in another authoritative source, the CRC Handbook of Chemistry and Physics, and accordingly the K value reported in the 1989 Merck Index demonstrates that the pK_a of lactic acid was known to be 3.86 prior to the filing date of the present application.

Third, the Examiner has alleged that the discrepancy between pK_a values between the 2006 Merck Index and the two references identified by the Examiner suggests controversy in the art concerning the pK_a value of lactic acid, and that in the face of such controversy one would adopt the lower pK_a value. Office Action, page 24. However, nothing in the references

acknowledges the existence of any such controversy or suggests that the discrepancy could result from anything other than repetition of a typographical error. In the face of a discrepancy one would naturally turn to an authoritative source for resolution. Applicants believe that the Merck Index and CRC Handbook of Chemistry and Physics are so well-known as authoritative sources of chemical data that no evidence of should be required to show that they are regarded as authoritative. Nonetheless, to advance prosecution Applicants present excerpts from editorial reviews to show that these publications have long been established as authoritative sources in the area of chemical data.

Excerpts from editorial reviews of the Merck Index are as follows:

> "The Merck Index was first published in 1899, and it will continue to serve as the standard reference for chemists, biochemists, pharmacologists, pharmacists, and other health professionals." (Journal of Medicinal Chemistry, February 8, 2007)
>
> "...the quality of the contents in one concise volume makes TMI the premier work of its kind...should be available as part of the arms-reach searching armamentarium of laboratory scientists of many stripes..." (Journal of Chemical Information and Modeling, March 2007)
>
> "Scientists working in many different areas...will be looking forwards to the future editions with their continued tradition of excellence." (American Journal of Therapeutics, September/October 2007)
>
> "...a must for academic, public, and special libraries...essential." (CHOICE, June 2007)

See http://www.amazon.com/Merck-Index-Encyclopedia-Chemicals-Biologicals/dp/091191000X (retrieved October 20, 2010).

Excerpts from editorial reviews of the CRC Handbook of Chemistry and Physics are as follows:

> This famous handbook continues to provide current, critically evaluated chemical and physical data in a one-volume format. A goldmine of information....(JACS, Vol. 127, No. 12, 2005)

> A standard text in libraries everywhere...this resource is an invaluable source of data. (Medical Reference Services Quarterly, Vol. 24, No. 3, Fall 2005)
>
> The CRC Handbook has been, in successive editions, on my bedside table for the last 55 years. (Oliver Sacks, Neurologist and author)
>
> This well-established publication from CRC Press has been given a face lift .... The publisher's philosophy, however, 'to provide broad coverage of data commonly encountered by physical scientists and engineers,' remains unchanged. (Prof. John Yates, Chemical Engineering Research and Design, May 2006)

See http://www.amazon.com/CRC-Handbook-Chemistry-Physics-88th/dp/0849304881 (retrieved October 20, 2010). Thus, it is clear that the Merck Index and the CRC Handbook of Chemistry and Physics are well accepted as authoritative sources of chemical data such as pK_a values. Because these authoritative sources are in agreement that the pK_a value for lactic acid is 3.86 and there is no evidence of any scientific controversy in the field concerning its value, one of ordinary skill in the art would have adopted this value and have disregarded the values reported in the Griffin and Van Ooijen references as plainly erroneous.

Response to rejections concerning Lactic Acid

Due to their recitation of lactic acid, claims 44-46, 50-51, 55, and 58-59 were previously rejected as allegedly obvious over Lawlis in view of Ward, Chang, and Van Ooijen (Office Action, page 23, and claim 57 was rejected in further view of Wangoh and EMBL AJ131677 (Office Action, page 25). The present amendment removes the recitation "lactic acid" from the Markush group in claim 44 and instead presents it in new **claim 79** and accordingly the rejections are now discussed with reference to this claim. Applicants respectfully traverse and request reconsideration and withdrawal of the rejection for the reasons stated in their previous response and for the further reasons described below.

Lawlis does not expressly mention lactic acid but does recite using the method with organic acids having between 1 and 5 carbon atoms (*see, e.g.*, Lawlis, claim 1). The Examiner

has alleged that one of ordinary skill in the art would have found it obvious to use lactic acid (which has 3 carbons) with the Lawlis method of killing cells, and within the range of pH values recited in the claim 44, to kill the cells of Ward. Specifically, because the Examiner had concluded that the pK_a of lactic acid was understood in the art to have been 3.08 at the time of filing, the rejection was based on an allegation that one of ordinary skill in the art would have found it obvious to use lactic acid with the Lawlis method at a pH of 1.08. However, Applicants have presented ample evidence that the pK_a of lactic acid was and still is accepted to be 3.86, notwithstanding typographical errors in the two non-authoritative sources that were identified by the Examiner. Accordingly, the pH value that would be used for lactic acid with the Lawlis method would have been 1.86, rather than 1.08 as alleged by the Examiner. This value is above the claimed pH range (which is between 1.7 and 1.0 in claim 79).

The Examiner has further alleged that one of ordinary skill in the art would have found it obvious to lower the pH to below pK_a - 2 to achieve greater cell kill. Office Action, page 24. However, as discussed above with acetic acid, Lawlis teaches that the pH value need only be lowered to pK_a - 2, *i.e.*, 1.86 for lactic acid, and further lowering of pH below this value is inconsequential. If greater cell killing is desired, Lawlis teaches that organic acid concentration is the critical result-effective value once the pH has been lowered to pK_a - 2. Once this pH value has been reached, 99% of the organic acid is in the active form, such that even the most extreme further lowering of pH could only increase the active acid concentration by about 1%. In contrast, active acid concentration increases proportionately to total organic acid concentration, and accordingly the active acid concentration can readily be doubled, tripled, or even further increased by simply increasing the organic acid concentration. Thus, once the pK_a - 2 threshold has been reached, the organic acid concentration is the critical result-effective variable. Accordingly, contrary to the alleged basis of rejection, one would not be motivated to increase cell killing by lowering the pH below pK_a - 2 because this would be ineffective; rather, if greater cell killing were desired then the organic acid concentration would be increased without altering pH.

Because acid concentration (and not pH) is the result-effective variable once the pH has been lowered to pK_a - 2, Lawlis does not provide any justification for lowering the pH any lower than 1.86 when using lactic acid. Moreover, Lawlis only teaches a method of killing cells and does not provide any guidance for selection of a pH value to achieve the beneficial result recited

in the present claims (inactivating unwanted glucoamylase activity while maintaining chymosin activity). The secondary references fail to remedy this deficiency of Lawlis. Therefore, there is no justification for lowering the pH to between 1.7 and 1.0 when using lactic acid with the Lawlis method, and accordingly claim 79 is not obvious.

Response to rejections concerning Formic Acid

Claims 60-62, 66-71, and 74-77 have been rejected as allegedly obvious over Lawlis in view of Ward (Office Action, page 18), and claim 73 has been rejected in further view of EMBL AJ131677 and Wangoh (Office Action, page 18). Though the rejected claims recite an inorganic acid, the Examiner has stated that these claims use an open transitional phrase and therefore are open to the presence of formic acid in addition to the inorganic acid. Based on this interpretation, the Examiner has alleged that it would have been obvious to use the method of Lawlis, with formic acid, and within the range of pH values recited in the present claims, to kill the cells taught by Ward. Applicants respectfully traverse and request reconsideration and withdrawal of the rejection for the reasons stated in their previous response and for the further reasons described below.

Lawlis teaches that the pK_a of formic acid is 3.75 and that cell killing is accomplished by lowering the pH to pK_a - 2. The Examiner has alleged that since Lawlis teaches using pH 1.75 with formic acid, one of ordinary skill in the art would have found it obvious "to adjust the pH of a medium with a mineral acid to 1.7 using only routine experimentation." Office Action, page 15. However, routine experimentation must have a purpose. Lawlis only teaches a method of killing cells, and this purpose does not justify lowering the pH below 1.75 with formic acid. Rather, if greater cell killing is desired, Lawlis teaches that organic acid concentration is the critical result-effective value once the pH has been lowered to pK_a - 2. Once this pH value has been reached, 99% of the organic acid is in the active form, such that even the most extreme further lowering of pH could only increase the active acid concentration by about 1%. In contrast, active acid concentration increases proportionately to total organic acid concentration, and accordingly the active acid concentration can readily be doubled, tripled, or even further increased by simply increasing the organic acid concentration. Thus, once the pK_a - 2 threshold has been reached, the organic acid concentration is the critical result-effective variable. Accordingly, contrary to the alleged basis of rejection, one would not be motivated to increase

cell killing by lowering the pH below pK_a - 2 because this would be ineffective; rather, if greater cell killing were desired then the organic acid concentration would be increased without altering pH.

For the foregoing reasons, Lawlis does not provide any justification for lowering the pH any lower than 1.75 when using formic acid. The secondary references fail to remedy this deficiency of Lawlis. Therefore, there is no justification for lowering the pH to between 1.7 and 1.0 when using formic acid with the Lawlis method, and accordingly claim 60 is not obvious. The remaining claims all properly depend from claim 60 and accordingly are not obvious for at least the same reasons.

CONCLUSIONS

Applicant submits that these amendments and arguments overcome all of the rejections as stated in the Office Action and places the pending claims in condition for allowance. Should any issues remain to be discussed in this application, the undersigned may be reached by telephone. Please charge any fees due for consideration of this paper, including fees for extension of time, to the undersigned's Deposit Account No. 50-0206.

Respectfully submitted,

HUNTON & WILLIAMS LLP

Dated: 10/26/10 By: Robin L. Teskin

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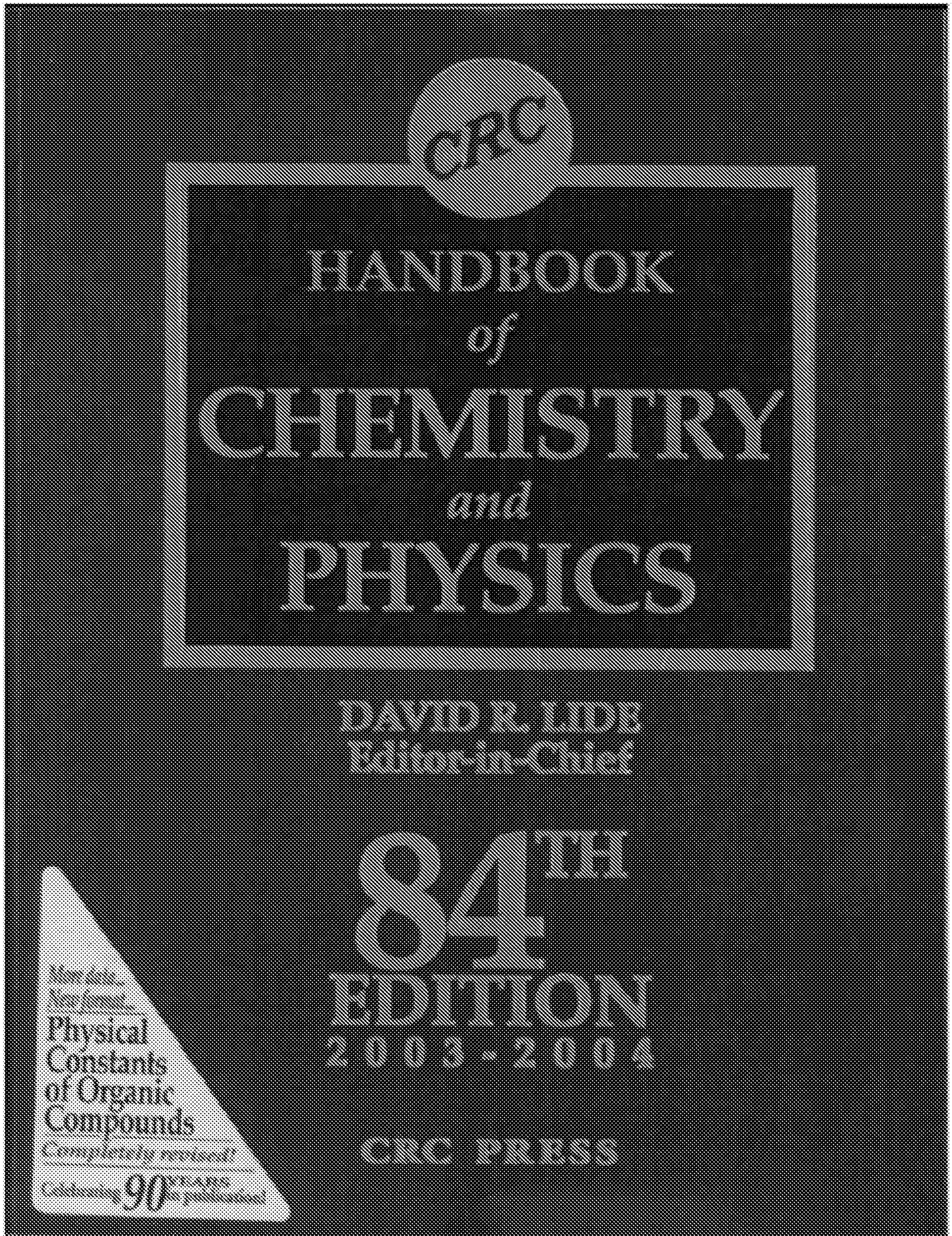
Exhibit A

Lide, D. R. The CRC Handbook of Chemistry and Physics. CRC Press (2003).


CRC
HANDBOOK
of
CHEMISTRY
and
PHYSICS
DAVID R. LIDE
Editor-in-Chief
84TH
EDITION
2003-2004
CRC PRESS
Physical
Constants
of Organic
Compounds
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Mol. Form.	Name	Step	t/°C	pK_a
$C_2H_6O_7P_2$	1-Hydroxy-1,1-diphosphonoethane	1		1.35
		2		2.87
		3		7.03
		4		11.3
$C_3H_2O_2$	2-Propynoic acid		25	1.84
C_3H_3NO	Oxazole		33	0.8
C_3H_3NO	Isoxazole		25	-2.0
$C_3H_3NO_2$	Cyanoacetic acid		25	2.47
C_3H_3NS	Thiazole		25	2.52
$C_3H_3N_3O_3$	Cyanuric acid	1		6.88
		2		11.40
		3		13.5
$C_3H_4N_2$	*1H*-Pyrazole		25	2.49
$C_3H_4N_2$	Imidazole		25	6.99
$C_3H_4N_2S$	2-Thiazolamine		20	5.36
C_3H_4O	Propargyl alcohol		25	13.6
$C_3H_4O_2$	Acrylic acid		25	4.25
$C_3H_4O_3$	Pyruvic acid		25	2.39
$C_3H_4O_4$	Malonic acid	1	25	2.85
		2	25	5.70
$C_3H_4O_5$	Hydroxypropanedioic acid	1		2.42
		2		4.54
$C_3H_5BrO_2$	3-Bromopropanoic acid		25	4.00
$C_3H_5ClO_2$	2-Chloropropanoic acid		25	2.83
$C_3H_5ClO_2$	3-Chloropropanoic acid		25	3.98
$C_3H_6N_2$	3-Aminopropanenitrile		20	7.80
$C_3H_6N_6$	1,3,5-Triazine-2,4,6-triamine		25	5.00
C_3H_6O	Allyl alcohol		25	15.5
$C_3H_6O_2$	Propanoic acid		25	4.87
$C_3H_6O_2S$	(Methylthio)acetic acid		25	3.66
$C_3H_6O_3$	Lactic acid		25	3.86
$C_3H_6O_3$	3-Hydroxypropanoic acid		25	4.51
$C_3H_6O_4$	Glyceric acid		25	3.52
C_3H_7N	Allylamine		25	9.49
C_3H_7N	Azetidine		25	11.29
C_3H_7NO	2-Propanone oxime		25	12.42
$C_3H_7NO_2$	*L*-Alanine	1	25	2.34
		2	25	9.87
$C_3H_7NO_2$	β-Alanine	1	25	3.55
		2	25	10.24
$C_3H_7NO_2$	Sarcosine	1	25	2.21
		2	25	10.1
$C_3H_7NO_2S$	*L*-Cysteine	1	25	1.5
		2	25	8.7
		3	25	10.2
$C_3H_7NO_3$	*L*-Serine	1	25	2.19
		2	25	9.21
$C_3H_7NO_5S$	*DL*-Cysteic acid	1	25	1.3
		2	25	1.9
		3	25	8.70
$C_3H_7N_3O_2$	Glycocyamine		25	2.82
$C_3H_8O_2$	Ethylene glycol monomethyl ether		25	14.8
$C_3H_8O_3$	Glycerol		25	14.15
C_3H_9N	Propylamine		25	10.54
C_3H_9N	Isopropylamine		25	10.63
C_3H_9N	Trimethylamine		25	9.80
C_3H_9NO	2-Methoxyethylamine		25	9.40
C_3H_9NO	Trimethylamine oxide		20	4.65
$C_3H_{10}N_2$	1,2-Propanediamine, (±)	1	25	9.82
		2	25	6.61
$C_3H_{10}N_2$	1,3-Propanediamine	1	25	10.55
		2	25	8.88
$C_3H_{10}N_2O$	1,3-Diamino-2-propanol	1	20	9.69
		2	20	7.93
$C_3H_{11}N_3$	1,2,3-Triaminopropane	1	20	9.59
		2	20	7.95
$C_4H_4FN_3O$	Flucytosine			3.26
$C_4H_4N_2$	Pyrazine		20	0.65
$C_4H_4N_2$	Pyrimidine		20	1.23
$C_4H_4N_2$	Pyridazine		20	2.24
$C_4H_4N_2O_2$	Uracil		25	9.45
$C_4H_4N_2O_3$	Barbituric acid		25	4.01
$C_4H_4N_2O_5$	Alloxanic acid		25	6.64
$C_4H_4N_4O_2$	5-Nitropyrimidinamine		20	0.35
$C_4H_4O_2$	2-Butynoic acid		25	2.62
$C_4H_4O_4$	Maleic acid	1	25	1.92
		2	25	6.23
$C_4H_4O_4$	Fumaric acid	1	25	3.02
		2	25	4.38
$C_4H_4O_5$	Oxaloacetic acid	1	25	2.55
		2	25	4.37
		3	25	13.03
C_4H_5N	Pyrrole		25	-3.8
$C_4H_5NO_2$	Succinimide		25	9.62
$C_4H_5N_3$	2-Pyrimidinamine		20	3.45
$C_4H_5N_3$	4-Pyrimidinamine		20	5.71
$C_4H_5N_3O$	Cytosine	1		4.60
		2		12.16
$C_4H_5N_3O_2$	6-Methyl-1,2,4-triazine-3,5(2H,4H)-dione			7.6
$C_4H_6N_2$	1-Methylimidazol		25	6.95
$C_4H_6N_4O_3$	Allantoin		25	8.96
$C_4H_6N_4O_3S_2$	Acetazolamide			7.2
$C_4H_6O_2$	*trans*-Crotonic acid		25	4.69
$C_4H_6O_2$	3-Butenoic acid		25	4.34
$C_4H_6O_2$	Cyclopropanecarboxylic acid		25	4.83
$C_4H_6O_3$	2-Oxobutanoic acid		25	2.50
$C_4H_6O_3$	Acetoacetic acid		25	3.6
$C_4H_6O_4$	Succinic acid	1	25	4.21
		2	25	5.64
$C_4H_6O_4$	Methylmalonic acid	1	25	3.07
		2	25	5.76
$C_4H_6O_5$	Malic acid	1	25	3.40
		2	25	5.11
$C_4H_6O_6$	*DL*-Tartaric acid	1	25	3.03
		2	25	4.37
$C_4H_6O_6$	*meso*-Tartaric acid	1	25	3.17
		2	25	4.91
$C_4H_6O_6$	*L*-Tartaric acid	1	25	2.98
		2	25	4.34
$C_4H_6O_8$	Dihydroxytartaric acid		25	1.92
$C_4H_7ClO_2$	2-Chlorobutanoic acid			2.86
$C_4H_7ClO_2$	3-Chlorobutanoic acid			4.05
$C_4H_7ClO_2$	4-Chlorobutanoic acid			4.52

Exhibit B

Windholz, M. The Merck Index: An encyclopedia of chemicals and drugs. Merck & Co. (1989).

THE MERCK INDEX

AN ENCYCLOPEDIA OF
CHEMICALS, DRUGS, AND BIOLOGICALS

ELEVENTH EDITION

Susan Budavari, *Editor*
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RAHWAY, N.J., U.S.A.

1989

HGG	human gamma globulin
HGH	hun[illegible] hormone
His	hist[illegible]
HIV	hun[illegible] deficiency virus
HLA	hun[illegible] antigen
HMG-CoA	3-h[illegible]thylglutaryl c[illegible]
Houben	a G[illegible]tion of medicinal F[illegible]
Houben Weyl	*Ho[illegible] Methoden der Organischen Chemie*, a German collection of preparative methods in organic chemistry (Thieme)
HPLC	high performance (pressure, power) liquid chromatography
hr	hour
HSA	human serum albumin
Hse	homoserine
HSV	herpes simplex virus
HT	hydroxytryptamine (serotonin)
HTLV	human T lymphotropic virus see also HIV
Hyl	hydroxylysine
Hyp	hydroxyproline
i-	optically inactive by internal compensation as *i*-inositol; archaic for *meso-*
I	inosine
Ia	I-region antigen
IACR	International Association of Cancer Registries
IARC	International Agency for Research on Cancer
IARC Monographs	*IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*
ibid	(*ibidem*) at the same place
I.C.C.	Interstate Commerce Commission
ICFA	incomplete Freund's adjuvant (same as FIA)
ICSH	interstitial cell-stimulating hormone (same as LH)
idem	the same (author); plural: *eidem*, the same (authors)
IDP	inosine diphosphate
i.e.	(*id est*) that is
IEF	Isoelectric focusing
IF, IFN	interferon
i.g.	intragastric
Ig	immunoglobulin
I.G. Farben	*Interessengemeinschaft der Farbenindustrie. Aktiengesellschaft-* the German dye trust
IGF-1	insulin like growth factor I
IL	interleukin
Ile	isoleucine
i.m.	intramuscular
IMP	inosine 5′-monophosphate (inosinic acid)
incl	including
incompat	incompatibility
INN	International Nonproprietary Name
inorg	inorganic
insol	insoluble
intern	internal
Intl	International
i.p.	intraperitoneal
IR	infrared
Ir genes	immune response genes
ISO	Internal Organization for Standardization
isoln	isolation
ITP	inosine triphosphate or idiopathic thrombocytopenic purpura
I.U.	international unit
I.U.C.	International Union of Chemistry
I.U.P.A.C.	International Union of Pure and Applied Chemistry
i.v.	intravenous
Japan. Kokai	Japanese patent (unexamined)
Japan. pat.	Japanese patent
K	dissociation constant, equilibrium constant
°K	degrees Kelvin
Kb	kilobase
kcal	kilocalorie(s)
K cell	killer cell
kg	kilogram(s)
αKG	α-ketoglutarate
KLH	keyhole limpet hemocyanin
KPA	prourokinase, kidney plasminogen activator
l	liter
l-	*levo*(rotatory), the opposite of *d*, *q.v.*
L-	*levo* (in configurational sense only), the opposite of D, *q.v.*
Lac	lactose
LAD	lymphocyte-activating determinant
λ (lambda)	wavelength
LATS	long acting thyroid stimulator
lb	pound(s)
LC	Lethal Concentration; LC_{50}, a concentration which is lethal to 50% of the animals tested; liquid chromatography
LCM	lymphocyte choriomeningitis
LD	Lethal Dose; LD_{50}, a dose which is lethal to 50% of the animals tested
LDH	lactate dehydrogenase
LDL	low density lipoproteins
Leu	leucine
LH	luteinizing hormone (same as ICSH)
ln	natural logarithm
LNPF	lymph node permeability factor
loc. cit.	(*loco citato*) in the place cited
log	logarithm (common)
l.o.i.	limit of impurities
LPS	lipopolysaccharide
Lys	lysine
m	meter; given after mass number signifies metastable isomer
m-	*meta*
M	molar (concentration)
Mab, mAb	monoclonal antibody
MAC	maximum allowable concentration
MAF	macrophage activating factor
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
mass spec	mass spectrometry
max	maximum, maxima
Mb	myoglobin
MbO_2	oxymyoglobin
M.C.A.	Manufacturing Chemists Association (U.S.A.)
mcg	microgram
mCi	millicurie
M_D	molecular rotation $\frac{[\alpha]_D \times \text{mol wt}}{100}$
MDH	malate dehydrogenase
Me	methyl CH_3—
Me_2CO	acetone
MeOH	methyl alcohol
Mellor's	*Mellor's Comprehensive Treatise on Inorganic and Theoretical Chemistry* (Longmans)
mEq	milli-equivalent (0.001 of an equivalent)
Met	methionine
fMet	*N*-formylmethionine

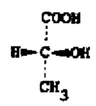
MetHb	methemoglobin
meV	millielectron volts
mfg, manuf	manufacturing
mfr	manufacture
mg	milligram
MHC	major histocompatibility complex
μCi	microcurie
μg	microgram
microcryst	microcrystalline
MIF	migration inhibition factor
min	minimum; also minute(s)
MIS	Mullerian inhibiting substance
misc	miscible
mixt	mixture
ml	milliliter (cubic centimeter)
MLD	minimum lethal dose
MLR	mixed lymphocyte reaction
mm	millimeter
mμ	millimicron(s), nanometer
mol wt	molecular weight
Monatsh.	*Monatshefte für Chemie*
mp	melting point: melts, melting at, when followed by a figure denoting temperature
M_r	relative molecular mass
ms-	*meso-* (internally compensated)
MS	mass spectrometry
MSH	melanocyte-stimulating hormone (melanotropin)
n	index of refraction (n_D^{20} for 20° and sodium light); normal, as *n*-propyl
N	normal (equivalents per liter, as applied to concentration); nitrogen (as in *N*-methylpyridine)
NAcneu	*N*-acetylneuraminic acid
NAD^+(NADH)	nicotinamide adenine dinucleotide (reduced form)
$NADP^+$(NADPH)	nicotinamide adenine dinucleotide phosphate (reduced form)
NANSAIDS	nonaspirin nonsteroidal anti-inflammatory drugs
NBS	National Bureau of Standards
NCTC	*National Collection of Type Cultures*
NDP	nucleoside 5′-phosphate
Neth. pat. **Appl.**	Netherlands patent application
N.F.	*National Formulary*
ng	nanogram (10^{-9} grams)
NGF	nerve growth factor
NIOSH	National Institute for Occupational Safety and Health
nm	nanometers
NMN^+(NMNH)	nicotinamide mononucleotide (reduced form)
NMP	nucleoside 5′-monophosphate
NMR	nuclear magnetic resonance
N.N.D.	*New and Nonofficial Drugs* (Lippincott, 1959-1964)
N.N.R.	*New and Nonofficial Remedies* (Lippincott, 1933-1958)
no.	number
nor-	(*Nitrogen ohne Radikal*) a prefix indicating a parent compound (no longer limited to nitrogenous compounds)
NRDC	National Research Development Corporation
NSAID	nonsteroidal anti-inflammatory drugs
NSC	National Service Center
NTP	nucleoside 5′-triphosphate
o-	*ortho*
O	denoting attachment to oxygen, as in *O*- acetylhydroxylamine

OA	ovalbumin
OAA	oxaloacetate
OD	optical density
op. cit.	(*opere citato*) in the work cited
org	organic
OSHA	Occupational Safety and Health Act
OsM	osmolar
oz	ounce(s)
P *or* p	concentration by weight (after optical rotations only)
p, pp	page(s)
p-	*para*
P_i	inorganic phosphate
Pa	pascal
PABA	*p*-aminobenzoic acid
PAF	platelet-activating factor
PAGE	polyacrylamide gel electrophoresis
passim	here and there, scattered
pat.	patent
PB report	Publication Board Report (United States Department of Commerce, Scientific and Industrial Reports)
PCA	passive cutaneous anaphylaxis
PCT	Patent Co-operation Treaty
PEP	phosphoenolpyruvate
petr, petrol	petroleum
PFC	plaque-forming cell
3PG	3-phosphoglycerate
PG	prostaglandin
PGA	pteroylglutamic acid (folic acid)
PGP	3-phosphoglyceroyl phosphate
pH	acid-base scale; log of reciprocal of hydrogen ion concentration
PHA	phytohemagglutin
Phe	phenylalanine
physiol	physiological
pK	log of the reciprocal of the dissociation constant, 1/log K
PKU	phenylketonuria
PMN	polymorphonuclear leukocyte
PP_i	inorganic pyrophosphate
ppm	parts per million
ppt(g)	precipitate, precipitating
pptd	precipitated
PQ	plastoquinone
Pr	propyl (normal)
prepd	prepared
prepn	preparation
press.	pressure
Pro	proline
PRPP	5-phosphoribosyl 1-pyrophosphate
ψ (psi)	pseudo
pt	point
PTH	parathyroid hormone
Q	coenzyme Q (ubiquinone)
q.q.v.	(*quae vide*) which see, plural
q.v.	(*quod vide*) which see
r	"roentgen" unit of radiation. That quantity of x or gamma radiation which produces one esu of charge in one cubic centimeter of air under standard conditions, i.e., the associated corpuscular emission per 0.001293 g of air (1 cc at 0° and 760 mm) produces, in air, ions carrying one esu
r-	racemic
R	alkyl, univalent hydrocarbon radical (or hydrogen)
(*R*)-	*rectus* (right). Absolute term describing the spatial arrangement about an asymmetric carbon when the observed order of decreasing priority of the groups is clockwise

35,000. Two types of subunits are distinguishable: M (muscle) type and H (heart) type. Lactate dehydrogenases of heart and muscle are mainly H_4 and M_4; all other possible hybrids have been found in various tissues. Elevations of lactate dehydrogenase activity have been found in myocardial infarction, hepatocellular necrosis, metastatic carcinoma, diabetic ketosis, sickle cell anemia, malignant lymphoma, infectious mononucleosis, and cerebral infarction: Standjord *et al., J. Am. Med. Assoc.* **182,** 1099 (1962). Comprehensive reviews: Everse, Kaplan, *Advan. Enzymol. Relat. Areas Mol. Biol.* **37,** 61 (1973); Holbrook *et al.,* in *The Enzymes,* vol. XI (part A), P. D. Boyer, Ed. (Academic Press, New York, 3rd ed., 1975) pp 191-292.

USE: In the determination of pyruvate (used in conjunction with reduced coenzyme). In the diagnosis of myocardial infarction and leukemia.

5214. D-Lactic Acid. *(R)-2-Hydroxypropanoic acid;* D(−)-lactic acid; levorotatory lactic acid; *l*-lactic acid; D-Milchsäure (German). $C_3H_6O_3$; mol wt 90.08. C 40.00%, H 6.71%, O 53.29%. Obtained by resolution of DL-lactic acid: Purdie, Walker, *J. Chem. Soc.* **61,** 754 (1892); Borsook *et al., J. Biol. Chem.* **102,** 449 (1933). Convenient laboratory prepn from glucose using *Lactobacillus leichmannii:* Brin, *Biochem. Prepn.* **3,** 61 (1953).

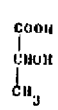


Crystals from ether + isopropyl ether, mp 52.8°. $[\alpha]_{546}^{21.5}$ −2.6° (c = 8). pK = 3.83. Sol in water, alcohol, acetone, ether, glycerol. Practically insol in chloroform.

Forms salts with many metals. Most of these salts are dextrorotatory.

Zinc D(+)-lactate, $Zn(C_3H_5O_3)_2.2H_2O$, crystals, $[\alpha]_D^{14}$ +8.18° (c = 2.5).

5215. DL-Lactic Acid. *2-Hydroxypropanoic acid;* racemic lactic acid; ordinary lactic acid; α-hydroxypropionic acid; Milchsäure (German); Lactovagan; Tonsillosan (Lösung). $C_3H_6O_3$; mol wt 90.08. C 40.00%, H 6.71%, O 53.29%. Occurs in sour milk as a result of lactic acid bacteria; also found in molasses due to partial conversion of sugars, in apples and other fruits, tomato juice, beer, wines, opium, ergot, foxglove, and several higher plants, especially during germination. Lactic acid is prepd technically by "lactic acid fermentation" of carbohydrates such as glucose, sucrose, lactose with *Bacillus acidi lacti* or related organisms such as *Lactobacillus delbrueckii, L. bulgaricus* etc. The fermentation is carried out at relatively high temps. Produced commercially by fermentation of whey, cornstarch, potatoes, molasses. Review on the production of lactic acid by fermentation: S. C. Prescott, C. G. Dunn, *Industrial Microbiology* (McGraw-Hill, New York, 3rd ed., 1959) pp 304-331. Chem prepns from acetaldehyde and CO in dil H_2SO_4 at 130-200° and 900 atm: Loder, U.S. pat. **2,265,945** (1938 to du Pont); by hydrolysis of hexoses with NaOH: Lock, U.S. pat. **2,382,889** (1943). Prepn of crystalline lactic acid: Borsook *et al., J. Biol. Chem.* **102,** 449 (1933). Toxicity data: Smyth *et al., J. Ind. Hyg. Toxicol.* **23,** 259 (1941).



Crystals, mp 16.8°. bp_{14-15} 122°; $bp_{0.5-1}$ 82-85°. K at 25° 1.38 × 10^{-4}. Heat of combustion at constant pressure 3615 cal/kg. Volatile with superheated steam. Sol in water, alc, furfurol; less sol in ether. Practically insol in chloroform, petr ether, carbon disulfide. *Pharm. Incompat:* Oxidizing agents, iodides, HNO_3, albumin. LD_{50} orally in rats: 3.73 g/kg (Smyth).

Barium salt, $C_6H_{10}BaO_6$, *barium lactate.* Powder. *Poisonous!* Sol in water, dil alcohol.

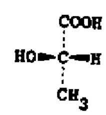
Copper salt dihydrate, $C_6H_{10}CuO_6.2H_2O$, *cupric lactate.* Green to blue crystals. Readily sol in water; practically insol in alcohol.

USE: In dyeing baths, as mordant in printing woolen goods, solvent for water-insoluble dyes (alcohol-soluble induline, nigrosine, spirit-blue); reducing chromates in mordanting wool; manuf cheese, confectionery; acidulant in beverages; for acidulating worts in brewing, for removing *Clostridium butyricum* in manuf of yeast; dehairing, plumping, and decalcifying hides; solvent for cellulose formate; flux for soft solder; manuf lactates which are used in food products, in medicine, and as solvents; plasticizer, catalyst in the casting of phenolaldehyde resins. *Caution:* Caustic in concd solns.

THERAP CAT: Acidulant.

THERAP CAT (VET): Has been used as a caustic, and in dilute solutions to irrigate tissues; as an intestinal antiseptic and antiferment.

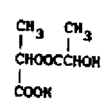
5216. L-Lactic Acid. *(S)-2-Hydroxypropanoic acid;* L(+)-lactic acid; dextrorotatory lactic acid; *d*-lactic acid; sarcolactic acid; paralactic acid; Fleishmilchsäure; L-Milchsäure. $C_3H_6O_3$; mol wt 90.08. C 40.00%, H 6.71%, O 53.29%. Occurs in small quantities in the blood and muscle fluid of man and animals. The lactic acid concn increases in muscle and blood after vigorous activity. L(+)-Lactic acid is also present in liver, kidney, thymus gland, human amniotic fluid, and other organs and body fluids. Obtained by resolution of DL-lactic acid: Purdie, Walker, *J. Chem. Soc.* **61,** 754 (1892); Borsook *et al., J. Biol. Chem.* **102,** 449 (1933). Convenient laboratory prepn from glucose by fermentation by *Lactobacillus delbrueckii:* Brin, *Biochem. Prepn.* **3,** 61 (1953). Prepn from hexoses using *B. dextrolacticus:* Andersen, Greaves, *Ind. Eng. Chem* **34,** 1522 (1942). Monograph: M. Brin, R. H. Dunlop, "Chemistry and Metabolism of L-and D-Lactic Acids", *Ann. N.Y. Acad. Sci.* vol. **119,** art. 3, 851-1165 (1965).



Crystals from acetic acid or chloroform, mp 53°. $[\alpha]_{546}^{21-22}$ +2.6° (c = 2.5). pK at 25°, 3.79. Forms salts with many metals. The salts are more sol in water than the salts of the racemic acid. Most of the salts are levoratory.

Zinc L(−)-lactate dihydrate, $Zn(C_3H_5O_3)_2.2H_2O$, prisms. $[\alpha]_D^{25}$ −8.2° (c = 2.5 in water).

5217. Lactic Acid Lactate. *2-Hydroxypropanoic acid 1-carboxyethyl ester;* 2-(lactoyloxy)propanoic acid; 2-(2-hydroxypropanoyloxy)propanoic acid. $C_6H_{10}O_5$; mol wt 162.14. C 44.44%, H 6.22%, O 49.34%. Prepd by heating lactic acid at 120° for 10 hours: Dietzel, Krug, *Ber.* **58,** 1307 (1925).



Pale yellow, clear, odorless oil. Sol in water and in the usual organic solvents.

Methyl ester, $C_7H_{12}O_5$. Prepn: Claborn, U.S. pat. **2,371,281** (1945 to the people of the U.S.). $bp_{7.4}$ 107°; n_D^{20} 1.4313.

USE: The methyl ester as a solvent or plasticizer.

5218. Lactobacillic Acid. *2-Hexylcyclopropanedecanoic acid;* 11,12-methyleneoctadecanoic acid; phytomonic acid. $C_{19}H_{36}O_2$; mol wt 296.48. C 76.97%, H 12.24%, O 10.79%. A lipid constituent of various microorganisms. Isoln from *Lactobacillus arabinosus:* K. Hofmann, R. A. Lucas, *J. Am. Chem. Soc.* **72,** 4328 (1950); from *Agrobacterium tumefaciens* and identity of phytomonic acid with lactobacillic acid: K. Hofmann, F. Tausig, *J. Biol. Chem.* **213,** 425 (1955). Structure: K. Hofmann *et al., J. Am. Chem. Soc.* **80,** 5717 (1958). Abs config: J. F. Tocanne, *Tetrahedron* **28,** 363 (1972).